


**THE EFFECTIVENESS OF
PROPHYLACTIC EYE DROPS ON THE
REVERSAL OF UV-LIGHT INDUCED
CATARACTS**

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A thesis Submitted in Partial Fulfillment of
Requirements of the CSU Honors Program

For Honors in the degree of
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Abstract:

Cataracts are a cloudiness of the lens within the eye which obstructs vision. They are found in many species including rats, dogs, and humans. Vision is hindered where this “cloudiness”, made of water build up and broken crystalline, proteins take form. Oculvet is an eye medication, used by veterinarians, containing carnosine as its active ingredient. Topical application of carnosine is reported to be an effective treatment for the prevention and removal of pre-existing cataracts. For this experiment, Sprague-Dawley rats were anesthetized and exposed to ultraviolet radiation, UVB and UVC. They were divided into four groups (n = 32), three of which had cumulative exposures of 5,495 minutes (Group A), 8,810 minutes (Group B), and 13,250 minutes (Group C). The control group received no UV exposure. After UV treatment, each experimental rat had one drop (0.05ml) of Clear Eyes[®] brand eye drops mixed with 1% carnosine administered to the left eye. The right eye was treated with Clear Eyes[®] brand eye drops alone. After four weeks of daily treatment, the rats were sacrificed and the lenses of both eyes were removed. Extent of cataract formation in the lenses of the treated and untreated eyes were compared. We found that there were no significant difference between the treated and untreated groups after administering the carnosine solution. However, there was a significant difference found within the treated and untreated subgroups. For example, there was a difference found between the treated lenses of Group A and the treated lenses of Group C. From this experiment, we found that the amount of time that the lens of the eye is subjected to UV light is directly correlated with the extent of cataract formation.

This experiment also shows that the active ingredient in Oculvet, carnosine, alone does not have a significant reversal effect on UV-light induced cataracts.

Introduction:

The eye is the primary photoreceptive organ in rats. It allows for better environmental awareness while seeking shelter, food, mates, water, etc. The basic components of the mammalian eye are illustrated in **Figure 1**. Each component builds on the others function, which enables the ultimate task of allowing for vision to occur.

Light, which comes from all directions, passes through the cornea. The cornea, which refracts light, allows both visible and ultraviolet light (UV) to pass through to the pupil. The iris is a ring of pigmented smooth muscle that controls the amount of light entering the eye by controlling the size of the pupil. The pupil is a circular opening in the center of the iris that passes the light to the lens of the eye. In addition to providing close focusing, the lens acts as a filter blocking all but the visible wavelengths. Once the light is refracted and filtered by the lens, it is passed to the retina where the light energy is transduced into electrical signals that the brain can understand.

The lens is made of transparent crystalline proteins. As stated above, the lens is capable of refraction, which allows for light to be focused on the retina. Refraction is the bending of rays of light when it is passed from one medium to another. A ray of light that enters into the lens is actually refracted twice, once when it enters the lens and once when it exits, shown using dotted lines in **Figure 2**. The amount of refraction that occurs as light travels between two media depends on the differences between the index refraction of the media. The lens of the eye accounts for nearly one-third of the eyes total refraction power. The cornea however holds most of the power accounting for nearly three-fourths of the total refraction.

A cataract has the ability to block the refraction of light. This is accomplished by causing a cloud-like film to form in the lens of the eye. This cloudiness is made up of a region of water build up and broken crystalline proteins caused by oxidative damage. Babizhayev et al. (2004) mentioned that the lens consists of about 65% water and 35% proteins. **Figure 3** is an example of the appearance of cataracts found in a Sprague-Dawley. Cataracts will not impair vision until they are large or thick enough to block the refracted light from getting to the retina. Progression of cataracts tend to be slow, often taking nearly a lifetime before completely disrupting vision (Chang and Gimbel 2004).

Cataracts are formed by a change in the chemical composition of the crystalline proteins within the lens. The change occurs when free radicals bond with oxygen molecules found throughout the body. The process of oxygen molecules bonding with other molecules is referred to as oxidation. Oxidation is an essential part of cataract formation. Babizhayev et al. in their 2002 paper, mention that cataractogenesis can be initiated by oxygen species and lipid peroxides that are produced within the crystalline lens. The formation of these elements has been accredited to oxidative stress within the lens.

UV light can be absorbed by oxygen, producing reactive oxygen species (ROS), including oxygen ions, free radicals, and peroxides. Some examples of ROS include, but are not limited to hydrogen peroxide, nitronium ion, nitric oxide, and superoxide anion (Pioro 2000). They are referred to as highly reactive species due to the fact that they have a pair of unshared electrons, which makes them unstable. These molecules seek other molecules with unpaired electrons that are capable of bonding with them to make them stable.

S. Williams et al. stated in their 2006 review that it is well known that antioxidants aid in decreasing oxidative stress. Antioxidants are chemicals that reduce the rate of oxidation by donating one of their electrons to a free radical so that it does not have to take electrons from other molecules. There are many different types of antioxidants and each has its own method of action. Some are naturally found in fruits and vegetables and are added to the body by ingestion. Examples of naturally occurring antioxidants include carnosine, vitamin A, vitamin C, and vitamin E. It should be noted here that antioxidants cannot stop all occurrences of oxidation since it is a naturally-occurring metabolic process.

Carnosine, a neuropeptide, is a dipeptide of beta-alanine and L-histidine found in skeletal muscle tissue, the brain, and in the lens of the eye (Babizhayev et al., 2002). Carnosine has been referred to as a “super antioxidant”, due to its reported ability to reduce the amount of oxidation within the body. If this research is valid, it has been reported that when carnosine is added to the eye in addition to what the body normally produces, it can protect the lens within the eye from further light damage. This protection blocks free radicals by placing a thicker than normal layer of carnosine between the eye and the direct source of UV light (Babizhayev et al., 2004). Carnosine has a variety of effect within the body ranging from prevention and reduction of cell damage to boosting immune properties.

Carnosine will bind to a molecule preventing it from becoming a free radical so that oxidation will not occur. This process is referred to as carnosinylation. In the absence of carnosine, the eye would experience normal free radical formation or even extensive free radical formation when introduced to UV light.

Ultraviolet light is electromagnetic radiation found between 1 and 380 nm wavelength. There are three main types of UV radiation; UV-C, UV-A, and UV-B. UV-C includes wavelengths less than 280 nm. This type of UV light is referred to as germicidal because of its ability to kill bacteria, viruses, molds, and fungi. UV-C radiation is the only type of solar radiation that is completely absorbed by the atmosphere. UV-A and UV-B can both reach the earth's surface, where they are capable of affecting organisms in different ways (Holick and Jenkins 2003).

UV-A light can be found between 315-380nm wavelengths. It is commonly referred to as "black light". This type of radiation is the cause of skin tanning, but not cataract formation. However, UV-B light is the ultraviolet light we are most interested in. UV-B includes wavelengths between 280-315 nm. It is suggested that this type of UV light can aid in the formation of cataracts (www.hvacpro.com, 2003).

In controlled experiments it has been shown that at a wavelength of 300 nm, (UV-B light) cataract formation will occur in Sprague-Dawley rats. Researchers found that at that wavelength maximum needed dose was 2.2KJ/M^2 (Soderberg et al., 2003). Other researchers found that 15 minutes of UV light exposure was the optimal exposure time, since exposures over 15 minutes did not increase the likelihood of cataract formation (Ayala et al., 2000). Ayala et al. (2002) also found that after one week of exposure the formation of cataracts seemed to have reached its maximum potential.

It is believed that by the age of maturity, 10 weeks for females and 12 weeks old for males, the eyes are fully developed and much more UV resistant than that of the eyes of still maturing rats (younger than 10 weeks). Lofgren et al. (2003) found that younger

rats, ages 3 and 6 weeks, are more sensitive to UV light than older, 17 and 52 week old rats, that are subjected to the same amount of UV.

In this study Sprague-Dawley eyes were exposed to UV light (UV-A: UV-B) in an effort to produce cataracts. Once a cataract had formed, the left eyes of all of the subjects were treated with a 1.0% carnosine solution. Carnosine has been suggested to protect against oxidative stress as well as increase a cells resistance to functional exhaustion which can lead to oxidation build up on the lens (Babizhayev et al., 2002). It was hypothesized that there would be no difference in the level of cataract formation based on different times of UV light exposure and that there would be no significant change in the level of cataract intensity after treatment of the lenses with carnosine solution.

This experiment was conducted under the supervision of Columbus State University (CSU) Professors Glenn Stokes (Dean of Science) and Kathleen Sellers (Assistant Professor of Biology). It was conducted at Benning Animal Hospital, with additional guidance from Dr. Lena Harris, the resident veterinarian.

Methods:

A total of 32 Sprague-Dawley rats (16 males, 16 females) were used in this experiment. All of the rats were obtained from Zivic distributing company (www.zivic.com). The rats were housed at Benning Animal Hospital in a room with a controlled air temperature, set to maintain a constant room temperature of 78°F, and photoperiod.

The rats were divided into four groups of eight, half male and half female. They were then separated based on ultraviolet radiation (UV light) exposure time. Each rat was housed in an individual cage and fed rat chow and water ad libitum (**Figure 4**).

Each group was exposed to UV light (300nm) for four sessions, spaced 3 days apart, over a two-week period. The time periods allotted for each rat's eye exposure to the UV light was 10 minutes (group A), 15 minutes (group B), and 20 minutes (group C) (**Table 1**). This accounted for 24 of the 32 rats. The remaining 8 rats were the control group. The control group received no UV light exposure. A Mineralight, model UVSL – 25 purchased from the Ultra-Violet Products Inc. was used for UV administration.

Rats were anesthetized ten minutes prior to UV light exposure using a glass bell jar filled with an isoflurane mixture. The isoflurane mixture contained four percent isoflurane with ninety-six percent propylene glycol. The eyes of the rat were then dilated using 0.5% tropicamide, 1 drop (0.5ml) per eye, at least 5 minutes before UV light exposure to ensure maximum exposure area (**Figure 5**).

After the four exposure periods, the rats were taken to an ophthalmologist to evaluate the cataract formation. No cataract formation was observed so the UV light source was changed to a germicidal light, also known to cause cataracts over an extended

time period. For this type of exposure, the rats did not have to be anesthetized (**Figure 6**). The rats were exposed to the germicidal light for a prolonged time period at first, starting with group (A) being exposed for 1,140 minutes, group B was exposed for 2,160 minutes, and group C was exposed for 2,880 minutes three times. This prolonged exposure appeared to be too long, and the rats were being burned. The areas with less dense hair growth were becoming red and inflamed which resembled sun burn. The areas that were mostly affected were the rats' ears and tail. Because of this, the rats were exposed to germicidal light source for a lesser time; 15 minutes for group A, 30 minutes for group B, and 60 minutes for group C for seventy-three exposure events. This led to a total accumulated time of: group A – 5,445 minutes, group B – 8,730 minutes, and group C – 13,140 minutes (**Table 1**). The rats were then re-evaluated by the same ophthalmologist, and it was reported that there was no observable cataract formation.

The light source was changed once more to a high pressure mercury lamp (100Watts). The rats were exposed for the following times: group A - 5 minutes, group B - 10 minutes, and group C – 15 minutes for two exposure periods. The rats were then re-evaluated by the same ophthalmologist, and it was reported that there were observable cataract formation. The total time of UV light exposure, using all three light sources totaled to the following: group A – 5,495 minutes, group B – 8,810 minutes, and group C – 13,250 minutes (**Table 1**).

The Bowman scale (**Figure 7**) was developed by the author to put a numeric value on the amount of light that was scattered as it passed through the lens. A value of Zero indicated no cataract formation; a value of one indicated slight cataract formation, indicated by slight light scattering; two indicated moderate cataract formation an example

is provided in **Figure 8**; and three indicated sever formation. Diagnosis and determination of the stages of cataract formation was assessed with the use of a compound microscope. This evaluation involved looking at the amount of reflected and refracted light as it passed through the lens. Light passing through the lens was refracted by the cataract producing a “star light” effect.

After the indirected UV exposure, the eyes were treated with an eye drop solution containing carnosine. For each rat, the left eye was treated with Clear Eyes ® brand eye drops mixed with a 1.0% solution of carnosine. The right eye was treated with Clear Eyes ® brand eye drops only, without carnosine. The eye drops were administered twice a day for four weeks. The eyes of the rats were held open with one hand while the eye drop was administered with the other, to ensure that the eye drop was applied into the eye.

As mentioned above, the eye drops used for this experiment was Clear Eyes ® brand eye drops containing: Naphazoline Hydrochloride (0.012%) which is a eye redness reliever and glycerin (0.2%) which is a lubricant as well as benzalkonium chloride (preservative), boric acid, edentate disodium (preservative), purified water, and sodium borate. After each rat's eyes had been treated with the eye drop solution for four weeks, they were re-evaluated via the Bowman scale to determine the progression of the reversal of the cataract.

At the end of this experiment, the rats were euthanized via barbiturate overdose, the lenses were removed, and each lenses was evaluated once more via the bowman scale to determine the extent of cataract formation. A mean was then found for each Group (**Table 2**).

Three statistical analysis tests were then used to determine the differences between the groups. A one-way ANOVA was used to find the differences among all the groups; Control, A, B, and C. A t-Test was used to find the differences between the treated and untreated lenses, and a Tukey HSD test was used to find the differences between each of the three groups (A, B, and C) for both treated and untreated lenses.

Results:

All of the following tests were performed based on the scores that were given to each set of lenses by the author. **Table 2** shows the mean scores, according to the Bowman scale (**Figure 7**) for both the treated and untreated eyes.

Using a t-Test for correlated samples we found that there was not a significant difference ($p = 0.17$) between the treated and untreated eyes of the control group (**Chart 1**). The standard deviations were found for each group, Control, A, B, and C; and for each subgroup, treated and untreated (**Table 3**). The difference between treated and untreated eyes for groups A ($p = 0.18$), B ($p = 37$), and C ($p = 0.17$) were also found to be non-significant.

A One-Way ANOVA was used to explore the differences among the exposure groups for the untreated and treated eyes (**Chart 1**). It found that there was a significant difference ($p = 0.0001$) between the untreated groups, Control, A, B, and C. The one-way ANOVA also found that there was a significant difference between all of the treated eye groups.

Using a Tukey HSD test, a significant difference between the treated and untreated sub groups for all four groups, Control, A, B, and C was determined. There was no significance found between the untreated eye groups of the Control and Group A, Group A and Group C, and Group B and Group C. However, a significant difference was found between the Control and Group B ($p < 0.01$), Control and Group C ($p < 0.01$), and Group A and Group C ($p < 0.05$).

Discussion:

From the results of this experiment, we found by using a t-Test, that there was no significant difference between treated and untreated lenses for all four groups; Control, A, B, and C. From the one-way ANOVA, we learned that there was no significant differences between the treated and untreated lenses, however, there were significant differences found between different exposure groups. The final test, the Tukey HSD test, found that the groups with similar lengths of exposure has the least amount of difference and those with the least similar lengths of exposure time has the most differences.

This research suggests that the length of time spent under UV-light does effect the formation of cataracts, and that there is no statistical evidence that carnosine alone has a reversal effect on UV-light induced cataracts. This is not to say that Ocluvet does not work, this is to say that carnosine alone, the active ingredient in Ocluvet does not reverse cataract formation when acting alone. It is possible that the other ingredients within the Ocluvet solution activate the carnosine in a way that makes it more effective. It is also possible that UV-light induced cataracts are harder to breakdown then naturally formed cataracts.

Some questions that may rise include: Are UV-induced cataracts harder to breakdown then naturally formed cataracts? And if so, why? Would a higher percent of carnosine in the eye drop solution make a difference in the results?

There was one main problem that was encountered while performing this experiment. The original light source used to induce cataract formation was not powerful enough to cause the formation to occur in the time suggested by the authors whose works

were cited in the introduction. The light source, as a result, had to be changed twice before finding a light powerful enough to achieve the desired results.

When trying to induce cataract formation, it is suggested that a high pressure Mercury lamp be used from the beginning. I would like to see more research done associated with this study. Future projects to be accomplished by myself or other individuals include:

- Continue to subject the rats to UV light while administering the prophylactic eye drops and record results.
- Use different brands of eye drops to see if they all have similar effects when carnosine is added.
- Increase the percentage of carnosine used from 0.1%.

Figure 1: Components of the eye.

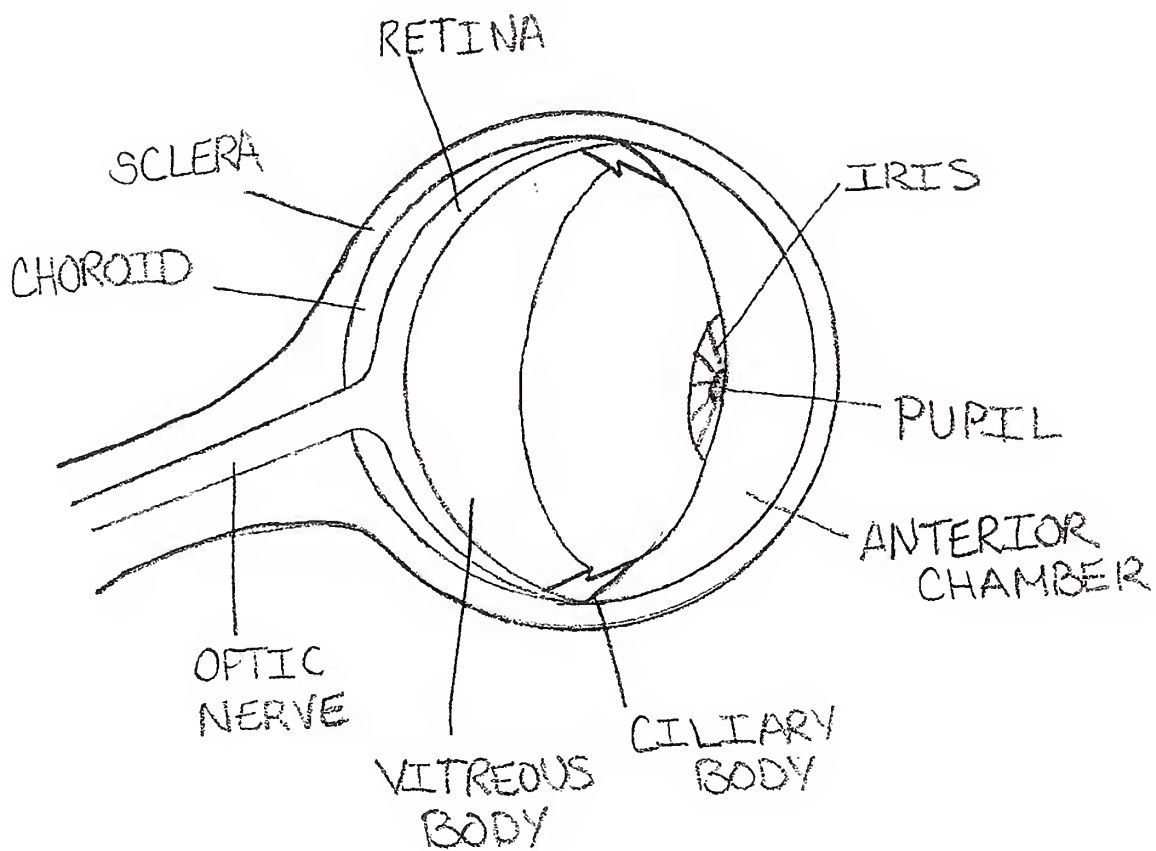


Figure 2: Refraction by the lens within the eye.

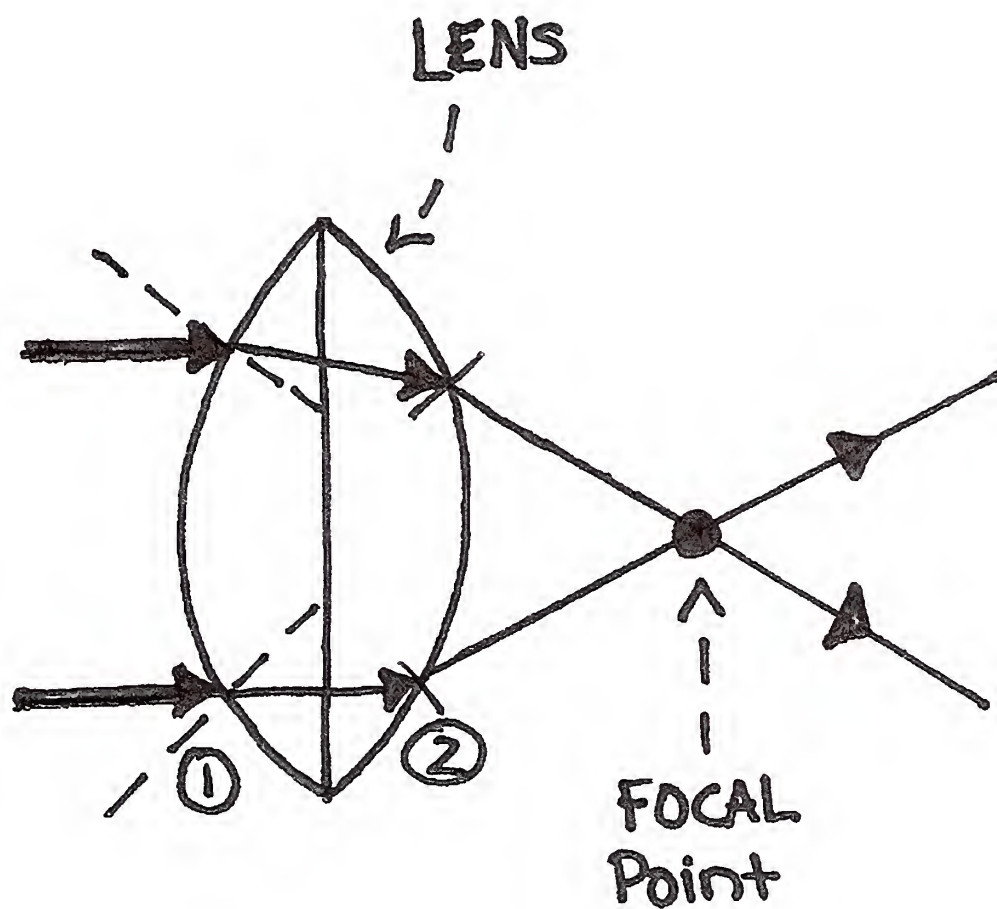


Figure 3: Image of how the cages were arranged.



Figure 4: A Sprague- Dawley with cataracts (Jenny, 1999).



Figure 5: Rats eyes being exposed to UV light (Mineralight – First light used)



Figure 6: Rats being exposed to UV light (Germicidal light – second light used)

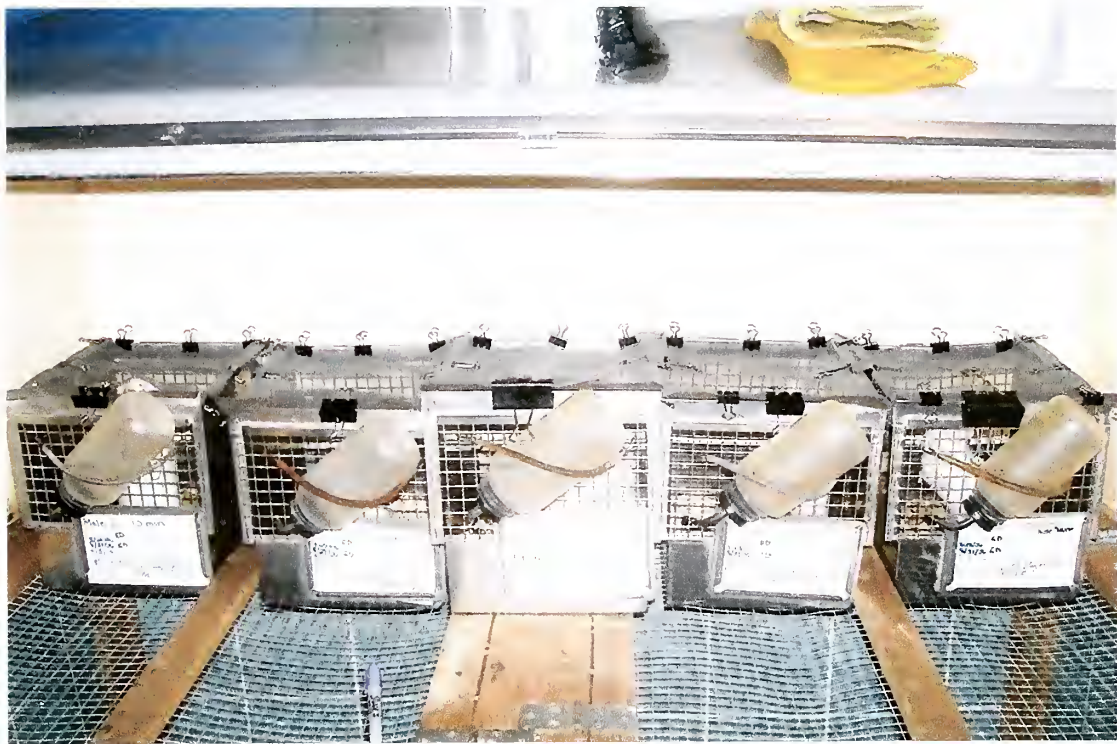


Figure 7: The Bowman Scale. Zero indicated no cataract formation, one indicated slight cataract formation, two indicated moderate cataract formation, and three indicated severe cataract formation.

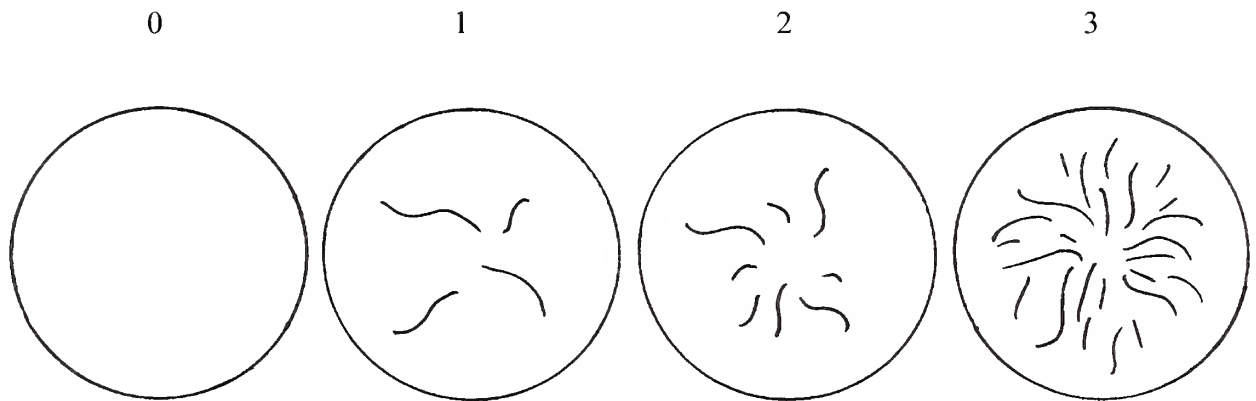


Figure 8: Lens of Sprague-Dawley Rats. This lens was scaled as a two on the Bowman Scale (shown above).

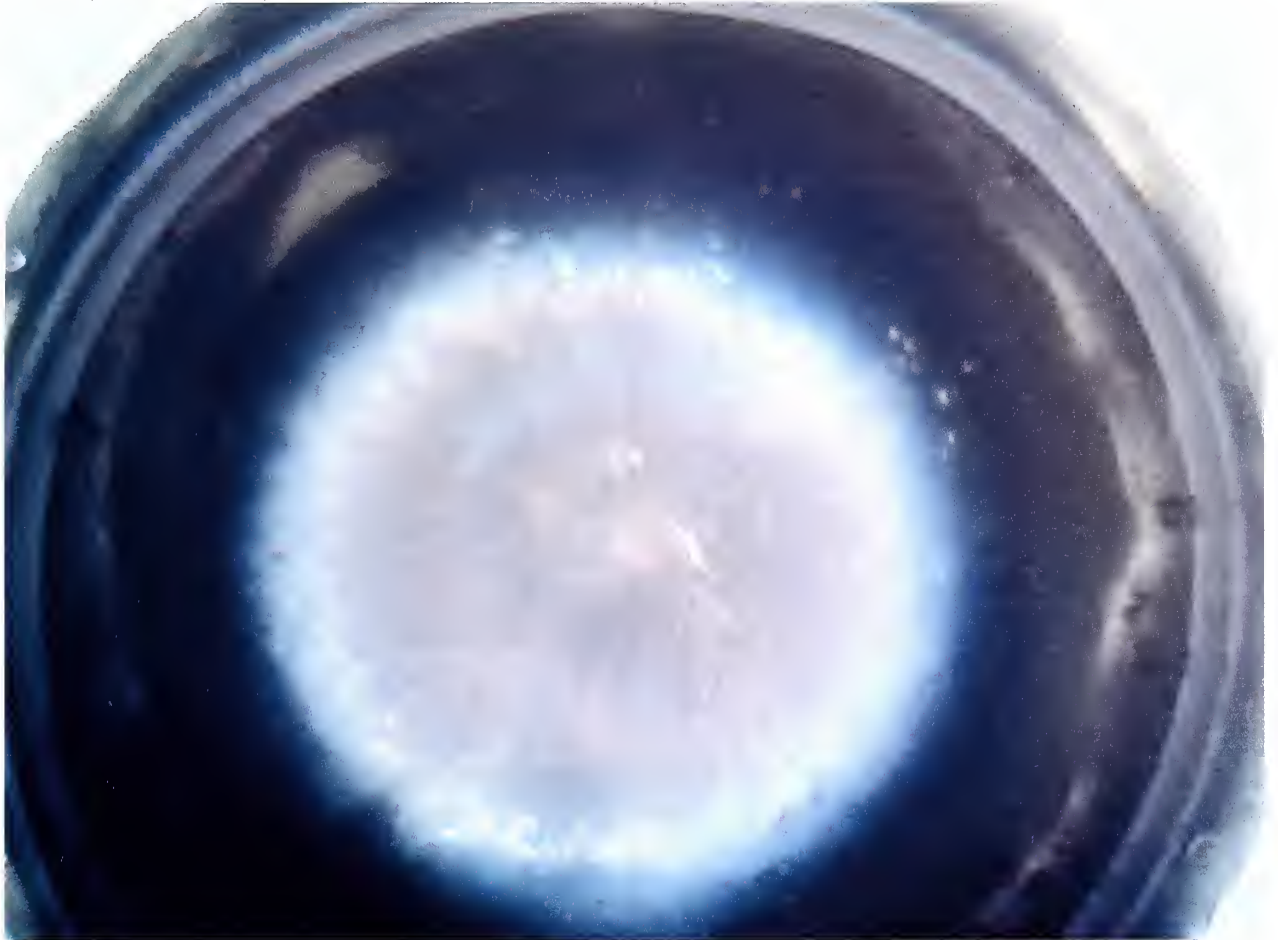
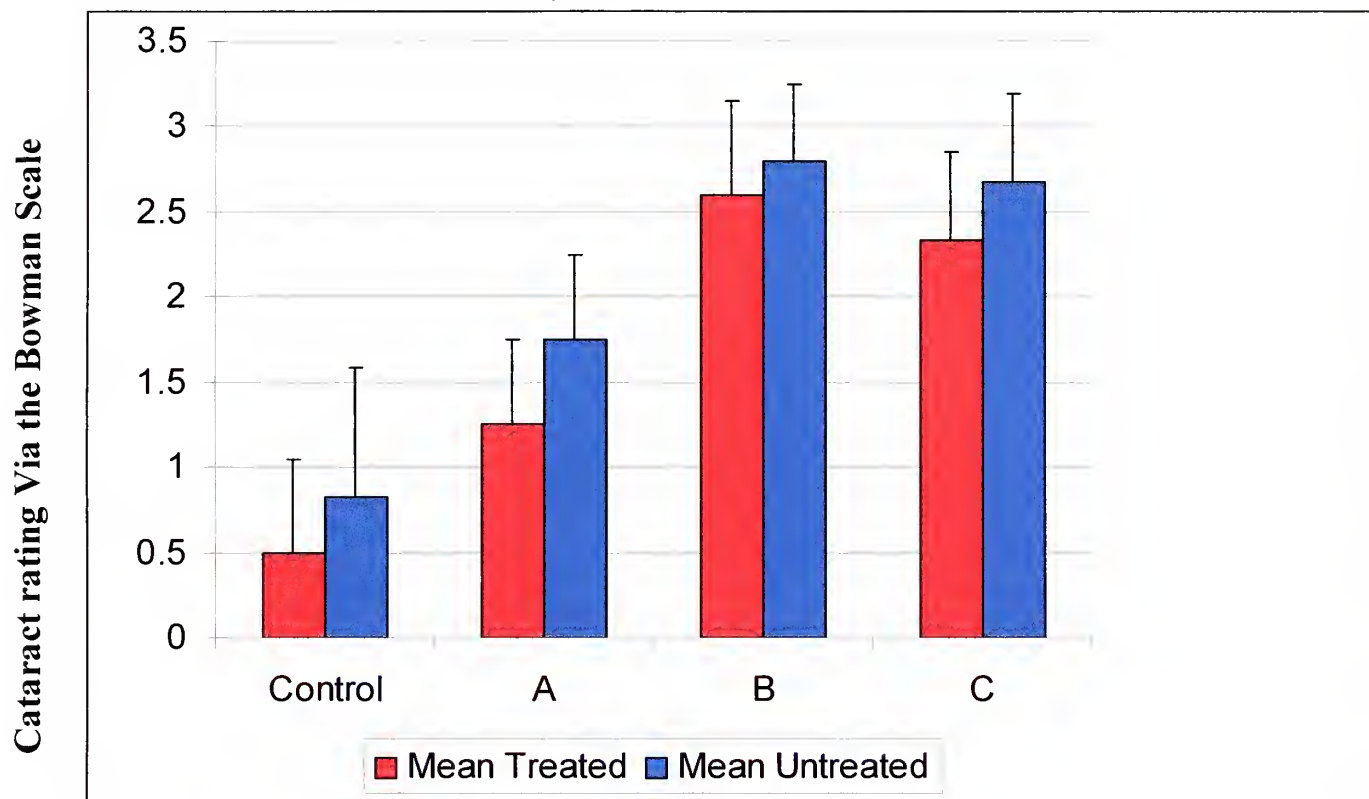


Chart 1: The relationship between the mean scores, using the Bowman Scale, of treated vs. untreated eyes.



Mean scored of Treated vs. Untreated eyes

Table 1: The sources of light used and the exposure time for each group in minutes and the total number of hours of exposure time for each group.

Type of light used	Number of minutes exposed		
	Group A	Group B	Group C
Mineral light	40	60	80
Germicidal Light	5445	8730	13140
High Pressure Mercury lamp	10	20	30
Total Minutes	5495	8810	13250
Total Hours	91.583	146.83	220.83

Table 2: The mean score for treated and untreated eyes for each group using the Bowman Scale.

Group	Mean Treated	Mean Untreated
Control	0.5	0.83
A	1.25	1.75
B	2.6	2.8
C	2.33	2.67

Table 3: Standard Deviations for both treated and untreated lenses for all groups.

Group	Treated (+/-)	Untreated (+/-)
Control	0.55	0.75
A	0.5	0.5
B	0.55	0.45
C	0.52	0.52

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